IN THE CLAIMS

Claims 1-75 (Canceled)

76. (Previously presented) A method of downregulating expression of a target gene in an RNA stress response-competent cell, comprising:

introducing into the cell an expression vector encoding a double stranded RNA corresponding to the target gene such that said double stranded RNA is expressed and expression of said target gene is specifically downregulated,

wherein intracellular expression of said double stranded RNA in said stress response-competent cell does not induce a detectable RNA stress response.

77. (Previously presented) The method of claim 76, wherein transfection of the same double stranded RNA produced outside the cell induces an RNA stress response in the RNA stress response-competent cell.

78. (Previously presented) The method of claim 76, wherein said target gene is an endogenous gene.

79. (Previously presented) The method of claim 78, wherein said target gene is a pathogen gene.

80. (Previously presented) The method of claim 76, wherein said stress-competent cell is a vertebrate cell.

81. (Previously presented) The method of claim 80, wherein said vertebrate stress-competent cell is a mammalian cell.

82. (Previously presented) The method of claim 76, wherein said double stranded RNA is expressed as a single transcript that contains an inverted repeat.

83. (Previously presented) The method of claim 76, where said double stranded RNA is formed from two separate transcripts expressed from two promoters.

84. (Previously presented) The method of claim 83, wherein said double stranded RNA is transcribed from the same nucleic acid sequence using two convergent promoters.

85. (Previously presented) The method of claim 77, wherein the region of the double stranded RNA that is present in double stranded conformation includes at least 30 nucleotides.

86. (Previously presented) The method of claim 85, wherein the region of the double stranded RNA that is present in double stranded conformation includes at least 50 nucleotides.

87. (Previously presented) The method of claim 86, wherein the region of the double stranded RNA that is present in double stranded conformation includes at least 75 nucleotides.

88. (Previously presented) The method of claim 87, wherein the region of the double stranded RNA that is present in double stranded conformation includes at least 100 nucleotides.

89. (Previously presented) The method of claim 88, wherein the region of the double stranded RNA that is present in double stranded conformation includes at least 200 nucleotides.

90. (Previously presented) The method of claim 76, wherein expression of said target gene is decreased at least 50%.

91. (Previously presented) The method of claim 90, wherein expression of said target gene is decreased 90%.

92. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected by testing for nicked DNA using a TUNEL assay.

93. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected by testing for activation of protein kinase R (PKR).

94. (Previously presented) The method of claim 93, wherein PKR activation is detected by testing for phosphorylation of EIF2alpha.

95. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected by testing for induction and/or activation of 2'5'oligoadenylate synthetase (OAS).

96. (Previously presented) The method of claim 95, wherein activation of 2'5'OAS is detected by ribosomal RNA fragmentation.

97. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected by testing induction and/or activation of interferon alpha, beta or gamma.

98. (Previously presented) The method of claim 97, wherein induction of interferon alpha, beta or gamma is detected using an ELISA assay.

99. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected microscopically by looking for one or more cytopathic effects.

100. (Previously presented) The method of claim 99, wherein said one or more cytopathic effects are selected from the group consisting of detached cells, rounded cells, increased vacuoles and morphological changes, in comparison to untreated cells.

101. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected by measuring the division rate of the transfected cells as compared to untreated cells.

102. (Previously presented) The method of claim 76, wherein an RNA stress response or lack thereof is detected by assaying for apoptosis.

103. (Previously presented) The method of claim 102, wherein said apoptosis assay is selected from the group consisting of reduction of MTT tetrazolium dye, Annexin V staining, propidium iodide staining, DNA laddering, PARP cleavage, caspase activation and assessment of cellular and nuclear morphology.

104. (Previously presented) A method of identifying a double stranded RNA delivery complex formulation so as to minimize cytotoxicity associated with delivery of a double stranded RNA of interest, comprising:

(1) mixing said double stranded RNA with a positively charged delivery agent at two or more different positive/negative charge ratios to form at least two different delivery complex formulations comprising said double stranded RNA;

(2) adding said at least two delivery complex formulations to target cells; and

(3) monitoring said cells for signs of toxicity,

wherein a complex formulation is identified that minimizes cytotoxicity associated with delivery of said double stranded RNA.

105. (Previously presented) The method of claim 104, wherein the concentration of double stranded RNA is between 1 pg and 10 μg.

106. (Previously presented) The method of claim 105, wherein the concentration of double stranded RNA is between 10 ng and 10 μg.

107. (Previously presented) The method of claim 104, wherein the two or more different positive/negative charge ratios are between 0.1 and 1000.

108. (Previously presented) The method of claim 104, wherein the positively charged delivery agent is selected from the group consisting of cationic lipids, cationic surfactants and local anesthetics.

109. (Previously presented) The method of claim 104, further comprising a step wherein said cells are analyzed to determine if post transcriptional gene silencing (PTGS) was induced.

110. (Previously presented) The method of claim 109, wherein PTGS is detected by measuring protein levels.

111. (Previously presented) The method of claim 104, wherein said cytotoxicity is an RNA stress response.

112. (Previously presented) The method of claim 104, wherein said signs of toxicity are selected from the group consisting of nicked DNA, phosphorylation of EIF2alpha, induction and/or activation of 2'5'oligoadenylate synthetase (OAS), and induction and/or activation of interferon alpha, beta or gamma.

113. (Previously presented) A double stranded RNA complex formulation identified by the method of claim 104.

114. (Previously presented) The double stranded RNA complex formulation of claim 113, wherein the positively charged delivery agent is a cationic lipid, and the positive/negative charge ratio of lipid to double stranded RNA is greater than 10.

115. (Previously presented) A method for identifying a double stranded RNA that modulates a function in a vertebrate cell, comprising

(a) transfecting a population of vertebrate cells with a double stranded RNA expression library of nucleic acids, wherein the nucleic acids of said expression library are capable of forming double stranded RNA upon expression, and wherein at least two cells of said population of cells are each transfected with a single, different, chromosomally integrated nucleic acid from said double stranded RNA expression library;

(b) culturing said transfected cells until not more than five episomal vectors from said library remain in each of said transfected, cultured cells;

(c) allowing double stranded RNA to be expressed from said transfected nucleic acids; and

(d) assaying for modulation of said function in said transfected cells, wherein said assay identifies a cell expressing a double stranded RNA that modulates said function.

116. (Previously presented) The method of claim 115, wherein said transfected cells are cultured until no episomal vectors from said library remain in each of said transfected, cultured cells.

117. (Previously presented) The method of claim 115, wherein each chromosomally integrated nucleic acid from said double stranded RNA expression library is integrated randomly.

118. (Previously presented) The method of claim 117, wherein said randomly integrated nucleic acids are integrated using a retroviral expression library.

119. (Previously presented) The method of claim 115, wherein each chromosomally integrated nucleic acid from said double stranded RNA expression library is integrated at the same site in each cell.

120. (Previously presented) The method of claim 117, wherein said chromosomally integrated nucleic acids are integrated into a loxP site in the presence of Cre recombinase.

121. (Previously presented) The method of claim 115, wherein double stranded RNA is expressed in said transfected nucleic acids from an inducible promoter.

122. (Previously presented) The method of claim 121, wherein double stranded RNA is expressed using a tet ON/OFF system.

123. (Previously presented) The method of claim 115, wherein said function that is modulated is the biological activity of a target polypeptide.

124. (Previously presented) The method of claim 115, wherein said function that is modulated is expression of a target gene.

125. (Previously presented) The method of claim 115, wherein said function that is modulated is selected from the group consisting of increased or decreased cell invasion, motility, apoptosis, growth, differentiation, dedifferentiation, regeneration and the ability to support viral replication.

126. (Previously presented) The method of claim 115, said method further comprising

(e) identifying said nucleic acid by amplifying said nucleic acid and sequencing said amplified nucleic acid.

127. (Previously presented) The method of claim 115, wherein said double stranded RNA expression library comprises cDNA sequences.

128. (Previously presented) The method of claim 115, wherein said double stranded RNA expression library comprises random sequences.

129. (Previously presented) The method of claim 115, wherein said cell is a mammalian

cell.

130. (Previously presented) The method of claim 129, wherein said cell is a human cell.

131. (Previously presented) The method of claim 115, wherein said cell is selected from the group consisting of a cancer cell, a cell of the immune system, a stem cell, a neuronal cell, a muscle cell, and an adipocyte.

132. (Previously presented) The method of claim 115, wherein each nucleic acid is contained in a vector.

133. (Previously presented) The method of claim 115, wherein each nucleic acid is expressed from RNA polymerase II promoter, an RNA polymerase I promoter, an RNA polymerase III promoter, or a mitochondrial promoter.

134. (Previously presented) The method of claim 115, wherein the sense strand and the anti-sense strand of each double stranded RNA are transcribed from the same nucleic acid using two convergent promoters.

135. (Previously presented) The method of claim 115, wherein each nucleic acid comprises an inverted repeat such that upon transcription mRNA forms a double stranded RNA.

136. (Previously presented) The method of claim 115, wherein each double stranded

RNA is between 5 and 100 nucleotides in length.

137. (Previously presented) The method of claim 115, wherein each double stranded RNA is at least 100 nucleotides in length.

138. (Previously presented) The method of claim 115, wherein each double stranded RNA is at least 250 nucleotides in length.

139. (Previously presented) The method of claim 115, wherein each double stranded RNA is at least 500 nucleotides in length.

140. (Previously presented) The method of claim 115, wherein each double stranded RNA is at least 1000 nucleotides in length.

141. (New) The method of claim 77, wherein the region of the double stranded RNA that is present in double stranded conformation includes at least 20-25 nucleotides.